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ABSTRACT

We present the new and entirely mechanistic COSMO*perm* method to predict passive membrane permeabilities for neutral compounds, as well as anions and cations. The COSMO*perm* approach is based on compound specific free energy profiles within a membrane of interest from COSMO-RS (Conductor-like Screening Model for Realistic Solvation) calculations. These are combined with membrane layer-specific diffusion coefficients, for example, in the water phase, the polar head groups and the alkyl tails of biochemical phospholipid bilayers. COSMO-RS utilizes first-principle quantum chemical structures and physically sound intermolecular interactions (electrostatic, hydrogen bond and van der Waals). For this reason, it is unbiased towards different application scenarios, such as cosmetics, industrial chemical or pharmaceutical industries. A fully predictive calculation of passive permeation through phospholipid bilayer membranes results in a performance of $r^2 = 0.92$; $rmsd = 0.90$ $\log_{10}$ units for neutral compounds and anions, as compared to gold standard black lipid membrane (BLM) experiments. It will be demonstrated that new membrane types can be generated by the related COSMO*plex* method and directly used for permeability studies by COSMO*perm*.

INTRODUCTION

Biomembrane permeabilities are of general interest in the uptake and distribution of pharmaceutical agents, chemical toxicants and environmental pollutants. Small solutes can permeate the phospholipid membrane by a passive diffusion process, and driven by a concentration gradient between the aqueous regions at the two sides of a bilayer.[1,2]

The intrinsic permeability coefficient P is defined from the steady-state-flux $J_{ss} = -P\,\Delta c$ across the phospholipid bilayer with a particular concentration difference Δc. Transport of small solutes can be described by Overton's rule and a more quantitative approach which is known as the solubility-diffusion model.[2,3]

In Overton's concept, the phospholipid membrane is regarded as a homogeneous organic matrix acting as barrier for solute diffusion. According to this, solutes transfer from water to the membrane with a volume based partition coefficient $K = c_m / c_w$ with c_m being the concentration in the membrane and c_w the concentration in water, both in in mol/l units). Fick's first law can be formulated as the following expression, with a phospholipid bilayer thickness of $2L$ and a concentration difference Δc_m within the membrane phase, or Δc_w between the aqueous donor and acceptor compartments of a permeability system:

$$J_{SS} = -\frac{D}{2L}\Delta c_m = -\frac{D}{2L}K\Delta c_w \tag{1}$$

Thus, leading to the simplified expression:

$$P = \frac{DK}{2L} \tag{2}$$

However, permeability through the membrane is dominated by those parts that are the least attractive and thus impose a free energy barrier. It is therefore no surprise that pure membrane permeability does not correlate well with membrane partition coefficients;[3] even though it is quite often correlated with partition coefficients to bulk solvents (e.g., 1-octanol to water or 1,9-decanediene to water).[4] For this reason, existing models for membrane permeation use alkane solvents such as hexadecane as a surrogate to mimic the membrane core, which is supposed to be the main barrier in membrane permeation for most compounds.[3,5] However, the bilayer environment is highly anisotropic along the normal direction $\vec{z}$ to the membrane surface.[6] An inhomogeneous solubility-diffusion model was proposed by Diamond and Katz in 1974,[7] arriving at the following analytical expression for the steady-state-flux of solutes through a membrane:

$$J_{SS} = -\frac{c(L) - c(-L)}{\int_{-L}^{L}\left[\frac{1}{K(z)D(z)}\right]dz} \tag{3}$$

$$\frac{1}{P} = \int_{-L}^{L}\left[\frac{1}{K(z)D(z)}\right]dz \tag{4}$$

Here, $K(z)$ and $D(z)$ are local partition and diffusion coefficients, respectively, at the solute position z (with $z = 0$ being the center of a bilayer membrane and $z = \pm L$ the boundaries of the symmetrical membrane). The solute position can be, e.g., in the aqueous phase, the polar head groups or the alkyl tails of biochemical phospholipid bilayers. The **equation 4** assumes that the diffusion in the membrane interior can be described by the Nernst-Plank equation in the absence of activated processes.[6] Indeed, missing a systematic derivation in literature, we counterchecked these

equations and came to the conclusion that the Diamond-Katz model is in exact agreement with Fick's laws of diffusion, as long as local diffusion and partition coefficients can be defined.

The local partition and diffusion coefficients are not experimentally accessible. Typically, they are predicted using time-consuming molecular dynamics (MD) simulations, partially relying on supercomputing facilities; a few examples are listed in the references.[1,6,8,9] As an alternative, we present the new and entirely mechanistic COSMO*perm* method to predict passive membrane permeabilities. The COSMO*perm* approach is based on compound specific free energy profiles $\Delta G(z)$ within a biomembrane of interest from COSMO-RS (Conductor-like Screening Model for Realistic Solvation) calculations.[10,11] These free energy profiles are computed by the COSMO*mic* method,[12] which has been demonstrated to yield at least comparably reliable results for the free energies of neutral solutes in micellar systems as achievable with MD simulations; but at about 0.01 percent of the computational cost (i.e., typically within a few minutes on a standard laptop computer with available quantum chemical COSMO information).[13–15] The local free energies are complemented by membrane layer specific diffusion coefficients $D(z)$, again, applying COSMO-RS parameters.

COSMO-RS utilizes first-principle quantum chemical structures and physically sound intermolecular interactions (electrostatic, hydrogen bond and van der Waals), and the COSMO*perm* method is designed to predict the membrane permeability not only of neutral, but also of charged compounds right from the beginning. While there already exist different models to predict the permeability of neutral compounds,[3,5,16] to the best of our knowledge, there are no alternative systematical methods capable of predicting permeation rates of anions and cations.

Nevertheless, the permeation of charged compounds is of potential interest for the biophysical research of fatty acid anions,[5] uncouplers of phosphorylation,[17] antibiotics,[18] drugs,[19] and nutrients.[20] In addition, the COSMO*perm* model is not limited to specific membrane types, but any atomistic membrane structure at the liquid-crystalline state generated by the COSMO*plex* method can be applied.[21] COSMO*perm* can be seen as a further development of the COSMO*mic* method mentioned above. COSMO*mic* originally was only applicable for neutral compounds and has itself been extended to ionic solutes before, allowing for the prediction of membrane to water partition coefficients of neutral and ionic species with an accuracy of about 0.7 $\log_{10}$ units.[22,23] the correct physical treatment of the energy barrier of ions in the alkyl phase (i.e., the center of a bilayer membrane) has been handled by the Born energy contribution.

In this paper, the COSMO*perm* model is validated against experimental black lipid membrane (BLM) permeabilities, for neutral compounds, ionizable compounds and permanent ions. BLM experiments are considered as gold standard for determining permeability values, because they are free of artifacts that may accompany cell-line experiments such as metabolism, active transport or ion-trapping in lysosomes.[3]

MATERIALS AND METHODS

Experimental BLM Data. Black lipid membrane (BLM) permeability data are collected from the literature. The validation data compilation includes neutral compounds as well as ionic protonation states and permanent ions.

In short, two compartments of a Teflon chamber are separated by a septum in a BLM experiment. A planar lipid bilayer of a few nanometers in size is formed across a small hole (about 100 μm to 1.5 mm in diameter) in the septum, which is pre-coated by hexadecane or by phospholipids dissolved in hydrophobic, viscous solvents (e.g., decane).[24,25] Both compartments are filled by aqueous buffer solution maintaining the pH value of particular interest and are magnetically stirred. The two BLM compartments are accessible electrodes are placed into both compartments and voltage or chemical gradients are applied. This allows to measure conductivities of ions or of the corresponding pH dependence of conductivities at electrophysiological conditions. Analytical, chemical or radiotracer methods may determine fluxes along an applied chemical gradient for neutral compounds. For fast permeating compounds, special care has to be taken to avoid limiting effects due to the aqueous boundary layer and associated acid-base reactions.[26,27] More details are presented by the workgroups of Gutknecht *et al.*,[28] Xiang *et al.*,[29] and Pohl *et al.*,[30,31]

The dataset is shown in **Table 1**. The experimental sources for neutral compounds, anions and cations are listed in the table. If more than one experimental value was reported in the literature for the same compound, the value derived from the membrane type closest to the DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) reference was taken.

COSMO-RS. The conductor-like screening model for realistic solvation (COSMO-RS) is a combination of the dielectric continuum solvation model COSMO[32] with an efficient statistical thermodynamic model of pairwise molecular surface interactions,[10,11] using the surface polarization charge densities σ of solutes and solvents arising from quantum chemical COSMO calculations. As most important molecular interaction modes, electrostatics and hydrogen bonding

are considered. The less specific dispersive interactions are described to first order based on element specific surface energies.[33]

COSMO*mic*. The COSMO*mic* extension for inhomogeneous systems uses information about the structure of a membrane. The membrane structure is represented by a layered liquid system of varying composition per layer with respect to the COSMO polarization charge density distributions, i.e., the so-called σ-profiles.[12] The generation of the membrane structures is described in the next section.

COSMO*mic* calculates the free energies of solutes in such a layered liquid system by sampling over all relevant positions, orientations and conformations of the solute in this system, resulting in reliable predictions of membrane to water partition coefficients and free energy profiles of solutes. Herein, the orientations are provided by rotations around the polarity-based center of the compound, because as a side-condition the polar sites need to cross all parts of the membrane as part of the permeation process. No additional side-conditions are introduced (e.g., to prevent flip-flops): the compounds are allowed to occupy all thermodynamically reasonable rotational states, by default the rotational states are defined by a set of 162 orientations per conformer and layer. The local membrane to water partition coefficient $K(z)$ is in thermodynamic relationship to the spatial free energy $\Delta G_{\mathrm{mic}}(z)$, as calculated by COSMO*mic* by the sampling mentioned above:

$$K(z) = \exp\left(\frac{-\Delta G_{\mathrm{mic}}(z)}{RT}\right) \tag{5}$$

COSMO*mic* has a proven track to provide at least comparably reliable results for the free energies of neutral solutes in micellar systems as achievable with MD simulations, at a fraction of the

computational cost.[34] More recently, COSMO*mic* has been extended to ionic solutes, now allowing

the prediction of the phospholipid membrane to water partition coefficients of neutral and ionic

species with an accuracy of about 0.7 $\log_{10}$ units.[22,23] No special parameterization of COSMO*mic*

was required beyond the underlying COSMO-RS parameterization. Only for inclusion of ions, the

membrane dipole potential[35] needed to be derived from fitting of an appropriate functional form

with three adjustable parameters.

Virtual Membranes Systems. Two virtual membrane structures are used within this work

and compared to each other.

The first membrane structure is taken from atomic distributions of molecular dynamics (MD)

simulations. Herein, time-averaged atomic distributions of a classic phospholipid system (1,2-

dimyristoyl-*sn*-glycero-3-phosphocholine, abbreviated as DMPC) were kindly simulated

(CHARMM36 force field) and provided by Jakobtorweihen.[34] A DMPC membrane potential is

applied, where three adjustable parameters per computational level are fitted for an optimal

prediction of membrane to water partition coefficients.

The second structure is generated by a self-consistent and iterative variant of COSMO*mic*, called

COSMO*plex*. This novel method can simulate the self-organization of micelles and membrane

structures as a fast alternative to MD simulations, typically in only 0.01% of the computational

time. For details of the COSMO*plex* method, the reader is referred to the publication of Klamt *et

al*.[21] In this study, the COSMO*plex* method is used to generate artificial chlorodecane containing

phospholipid bilayers as an occasionally used experimental reference system for mitochondrial

membranes.[36] They show an increased permeability, as compared to classic phospholipid

membranes. The COSMO*plex* system is set up by using two conformer sets, DMPC and

chlorodecane, plus water; more in detail, making use of the "autobox" functionality of the COSMO*plex* software with standard parameters and activated segment directionality (DIRPLEX) to arrive at a reasonable initial guess for the membrane system. The converged layered membrane system is used as input for further COSMO*perm* calculations. In this COSMO*plex* membrane the scaled membrane potential of the chlorodecane/DMPC system is generated in a self-consistent way by COSMO*plex*.

More details on the membrane structures and the membrane potentials are listed in section S3 of the supporting information.

Permeability in Inhomogeneous Membranes. The permeability P is calculated according to the inhomogeneous solubility-diffusion model, which was deduced in very detail by Diamond and Katz, see **equation 4**.[7] The following initial equations assume a single compound or protonation state penetrating the membrane. In this case, the resistance $R(z) = 1/P(z)$ in a particular penetration depth z is defined in the following way:

$$R(z) = \frac{dz}{K(z)D(z)} \tag{6}$$

In this equation, $K(z)$ is the local membrane/water partition coefficient in [(mol/l)/(mol/l)], calculated by **equation 5**; $D(z)$ the local diffusion coefficient of solutes penetrating a membrane layer, typically in [m²/s]; and dz is the depth of a membrane layer in [m]. The overall steady-state permeability P is then calculated:

$$P_{\text{steady - state}} = \frac{1}{\Sigma_{\text{layers}}R(z)} \tag{7}$$

Prediction of Diffusion Coefficients. The missing link between the chemical potential provided by COSMO*mic* in **equation 5** and the kinetics in **equation 6** are the layer-specific diffusion coefficients $D(z)$. Briefly, they are calculated via COSMO-RS based parameters from entropic and enthalpic contributions of solute-solvent and solvent-solvent interactions. Here, the solute is the permeating molecular species and the solvent environment a particular membrane layer of depth z. The layer-specific diffusion coefficient prediction is completed by the COSMO surface area of the permeant, as the magnitude of the shear force is proportional to the area, and a temperature dependence according to the Stokes-Einstein hydrodynamic theory.[37] The diffusion model is fitted against bulk liquid diffusion coefficients collected from the literature ($n = 499$; $r^2 = 0.81$; $rmsd = 0.298$ ln units; $F = 447$), and used in a fully predictive manner within COSMO*perm*. More details are shown in section S1 of the supporting information.

In principle, the COSMO*perm* model is independent from the underlying diffusion model used, and more mechanistic diffusion models might replace this semi-empirical model in the future.

pH Dependent Protonation States. A search of the World Drug Index for 1999 suggested that 63 % of the listed drugs were ionizable. Therefore, the consideration of protonation states is one important aspect for the quantification of membrane permeation.

In our model, both the neutral species and all ionic species (i.e., protonation states) are allowed to participate in the penetration process at the same time with individual partition coefficients and diffusion coefficients. A mechanistic parallel resistor model is applied. This replaces the resistance of **equation 6** for the calculation of the steady-state permeability in **equation 7** by a combined resistance model, incorporating each particular molecular species X:

$$\frac{1}{R_{tot}(\text{pH})} = \Sigma \frac{1}{R^X(\text{pH})}. \tag{8}$$

The pH dependent resistance of either the neutral or ionized protonation species is available from:

$$R^X(\text{pH}) = \frac{1}{f^X(\text{pH})} \Sigma_{\text{layers}} \frac{dz}{K^X(z)D^X(z)}. \tag{9}$$

The particular fraction of the neutral or ionized species $f^X(\text{pH})$ is calculated from the ionization fraction in the aqueous buffer solution surrounding the membrane system, which is related to the chemical specific $\text{p}K_a$ values in aqueous systems. The individual dissociation constants of the possibly multiple protonation and deprotonation sites are calculated by COSMO*therm*, derived from free energy differences between the particular species.[38,39] The population of the individual states follows from the partition function over all protonation states. The corresponding expressions are presented in section S2 of the supporting information.

The model assumes that the membrane potential does not change significantly, and that protonation or deprotonation reactions do not take place inside the membrane: acid-base reactions are very unlikely to occur in the alkyl tail part of the membrane, which imposes the main resistance of the permeation process for ions.

Free Energy of Ions in the Membrane Center. The probability for ions to be present in the non-polar center of a bilayer membrane is usually very low. This is in accordance with free energy profiles of ionic systems, which have their free energy minimum mostly close to the

phospholipids polar head group region .[40] However, the proper quantification of the free energy of

ions in the membrane interior is mandatory for a mechanistic grasp of the penetration process.

Unfortunately, the classic COSMO-RS method has a known problem with respect to the exact

quantification of the free energy of ions in non-polar media, which usually is unimportant, given

that ions are anyway of negligible importance in such media. But, for the quantification of the

permeation of permanent ions through phospholipid membranes, it is necessary to use accurate

free energies. Fortunately, in non-polar solvents the dielectric continuum approach is well justified,

and the bare COSMO energies can be used in combination with a dielectric scaling factor. The

COSMO-RS dielectric energy (E_{diel}) is defined as half of the electrostatic interaction energies of

the ideally screened polarized solutes ($\varepsilon_S = \infty$) with their screening charges,[11] which needs to be

corrected to the alkyl chain environment ($\varepsilon_{alkyl} = 2.1$):[41]

$$\Delta G_{Born}^{ion} = \left[1 - \left(\frac{\epsilon_{alkyl} - 1}{\epsilon_{alkyl}}\right)\right] E_{diel} \tag{10}$$

The final free energy profile $\Delta G(z)$ is then obtained by correcting the COSMO*mic* free energy

$\Delta G_{mic}(z)$ (**equation 5**) in the following way for ions:

$$\Delta G(z) = \Delta G_{mic}(z) + p_{Born}(z)\left[\Delta G_{Born}^{ion} - \mu_{misfit}^{ion}\right]_{alkyl} \tag{11}$$

Here, μ_{misfit} is the original misfit contribution to the chemical potential of the ion at infinite dilution,

which is replaced by the Born energy in the alkyl chain environment. And $p_{Born}(z)$ is a weight

factor for the correction term, approaching zero at the region of the polar head groups holding

significantly larger ε values as compared to the alkane environment:

$$p_{\text{Born}}(z) = \exp\left[-f_{\text{Born}}\left(\frac{\epsilon(z) - \epsilon_{\text{alkyl}}}{\epsilon_{\text{alkyl}}}\right)^2\right] \qquad (12)$$

The factor of the Born correction f_{Born} is an empirical parameter (and fixed to a value of 3 throughout this manuscript from free energy considerations for typical ions, further details not shown). Thus, the factor is not dependent on the type of the permeating ion. This function is relatively steep, keeping in mind that this correction term shall just correct for the free energies of ions in the alkyl tails, whereas interactions between ions and more polar parts of the phospholipids are well predicted by COSMO-RS. All remaining parameters result consistently from mechanistic COSMO-RS calculations.

Software. The quantum chemical COSMO files are needed for all involved relevant conformers of all solutes and their corresponding ions (if applicable) in the penetration process. The software BIOVIA COSMO*conf* is used for full energy minimization and conformer generation, utilizing the quantum chemical TURBOMOLE software.[42,43] Membrane free energy profiles, diffusion coefficients, pK_a values in the water phase and layer-specific electrostatic parameters for the Born correction are calculated using the COSMO*perm* module of BIOVIA COSMO*therm* 2019.[44] The currently best performance is achieved with a full optimization of the molecular geometry using the def-TZVP basis set (in short: "TZVP" level) and subsequent COSMO single point calculations using the def2-TZVPD basis set with additional diffuse basis functions and a novel fine grid cavity (in short: "TZVPD-FINE" level).[45] All calculations are performed at both levels, TZVP and TZVPD-FINE. If not indicated otherwise, the presented values (incl. diagrams and tables) are calculated by the more accurate TZVPD-FINE level.

RESULTS AND DISCUSSION

Free Energy and Resistance Profile in Membrane Layers. Free energy profiles and local resistances of the caproic acid as a typical example system are shown in **Figure 1a and 1b**, respectively. The local resistance opposing permeability is obtained by a combination of local diffusion coefficients and the height of the energy barrier (**equation 9**). The free energy profiles and, thus, the resistance profiles deviate significantly for the neutral species (blue lines) and the ionized species (red lines). It becomes obvious that there are two parts of the membrane, which are responsible for the main resistance in the permeation process. The first one is located at the central alkyl part of the membrane for both protonation states. Here the energy barrier is considerably higher for the ionized species.

Phospholipid membranes are considered as almost impermeable for ions (without the presence of membrane transporter proteins, which are not considered in this work), as it is certainly true for caproic acid. This assumption is probably also true for most pharmaceuticals; nevertheless, examples of ions permeating through phospholipid membranes to a significant extent by the passive, diffusion-controlled mechanism are discussed below. The central alkyl part is considered as the main barrier because polar organic chemicals will find this region rather unattractive. Many published approaches simplify the permeation process by a classic solubility-diffusion model through an alkane phase, e.g., hexadecane;[3,27,31] or even by using the octanol-water partition coefficient as classical descriptor.[5] The latter correlates strongly with the partitioning of neutral compounds into phospholipid membranes.[46] However, a second barrier shows up in our

calculations, which is located at the interface between the zwitterions in the polar head group and water. This barrier can be explained due to unfavorable interactions between the permeant and the zwitterionic polar head group. For the neutral state, a second free energy barrier does not show up. But there is a local maximum of the resistance profile (at $z = 19$ Å), related to a reduction of the diffusion coefficients at this point. The possibility of different rate-limiting barriers for different kinds of solutes has also been considered by several investigators.[7,47,48,49] In more detail, hydrophobic solutes may be rate-limited by diffusion through the peripheral region of the bilayer, which is more polar and more structured.[5] Indeed, this might be confirmed by running calculations for highly hydrophobic alkanes (see discussion in the supporting information, chapter S4). For more hydrophilic solutes, this could be related to electrostatic interactions between the negatively charged phosphate group of DMPC and solute anions (or negatively polarized surface areas of the solute); or vice versa, between the positively charged cholin group of DMPC and solute cations (or positively polarized surface areas of the solute). Especially in case of very high membrane permeability, the second barrier may constitute the rate-limiting step; at this point, the mechanistic COSMO*perm* model would outplay the classic approaches. A selection of predicted free energy barriers is shown in **Figure 2** for aspirin, sulfasalazine and lincomycin, illustrating the potential importance of this second barrier. This barrier might even be the rate-limiting step for sulfasalazine.

For ionizable chemicals, the actual site of deprotonation and protonation reactions is still a matter of debate. The prevalent ionic species might pass the aqueous boundary layer (ABL) and then transform into the corresponding neutral species close to the membrane surface.[26] However, how far these deprotonation and protonation reactions reach into the membrane itself is yet unclear.

ABL-related effects will be investigated in further mechanistic studies related to the mechanistic COSMO*perm* model.

Permeability of Neutral Compounds. The correlation between experimental BLM permeabilities and predicted COSMO*perm* permeabilities (using the DMPC system as reference) is shown in **Figure 3** (neutral: black symbols). The statistical analysis listed in **Table 2** shows squared correlation coefficients of $r^2 = 0.91$ [0.81] and deviations of $rmsd = 0.84$ [1.06] log_{10} units to the experiment for neutral compounds at the TZVPD-FINE [TZVP] level. By correcting the values slightly for the linear regression slope and intercept, the deviations decrease to $rmsd = 0.69$ [0.98]. The value range ($-7.96 \ldots +1.70$ log_{10} units) covers ten orders of magnitude. Low molecular volume compounds with an atom count ≤ 5 (e.g., HF, HCl, HSCN, HNO_3;) are not part of the statistics, related to partially deviating permeation mechanisms.[3,16]

Overall, the more accurate TZVPD-FINE level is recommended for the predictive calculation of permeability values of neutral compounds. These results are in a similar range as derived from a published BLM model ($r^2 = 0.91$; $rmsd = 0.64$) utilizing $-$ mostly experimentally determined $-$ pp-LFER (poly-parameter Linear Free Energy Relationship) parameters.[3]

At a first glance, the slope deviates slightly from unity for the neutral dataset (1.3 and 1.2 at the TZVPD-FINE and TZVP level, respectively), which can be partially explained by the deviations to the experiment at the high permeability end. Here, the aqueous boundary layer (ABL) might be accounted for in a different way by the experiment and the calculations. The ABL is not considered as rate-limiting in most of the cases in our dataset, so the few surrounding pure water layers (~ 5 Å) are treated in the same way as layers containing phospholipids. Effects introduced by buffer components in the experiments might play an additional role, which are also not considered

by the model at the current stage. As will be shown below, the combined dataset of neutral compounds and anions – which covers a larger value range – leads to a slope and intercept close to unity, so it might not be necessary to correct for the slope.

Permeability of Anions. The advantage of the COSMO*perm* model is the simultaneous handling of neutral and ionic species, without any recalibrations of the underlying COSMO-RS or COSMO*mic* method. The correlation between BLM permeability values and predicted COSMO*perm* permeability values is shown in **Figure 3** (anions: blue symbols), and the corresponding statistics listed in **Table 2**. The value range (-11.5 … $+2.2$ $\log_{10}$ units) covers fourteen orders of magnitude.

A squared correlation coefficient of $r^2 = 0.94$ [0.89] and a deviation of $rmsd = 1.00$ [1.40] $\log_{10}$ units is obtained for ions at the TZVPD-FINE [TZVP] level. Slight corrections for slope and intercept improve the $rmsd$ values to 0.93 [1.26]. Not part of the statistics are ions with only an upper threshold as the experimental information, but without knowledge of the exact value. The Born correction (**equation 11**) is applied in all TZVP calculations, which is already implicit part of the TZVPD-FINE parameterization (details not shown).

At the TZVPD-FINE level, predictions for compounds prone to strong push-pull effects are corrected by a constant offset of -2.5, as indicated in **Table 1**. These strong push-pull effects are characterized by strong deactivating groups (i.e., $-NO_2$, $-C{\equiv}N$, $-CO_2R$; with both $-I$ inductive and $-M$ resonance effects) *directly conjugated* to extremely activating groups (i.e., $-O^-$ anion). This is not a limitation of the COSMO*perm* method or COSMO-RS theory as such, but of the underlying quantum chemical calculation method, i.e. density functional theory, which tends to overestimate charge separation effects.[50] The TZVP results apparently are less sensitive to this

overestimation and the application of this correction term turned out to be needless due to fortunate error cancellation.

The statistics of the TZVP level is significantly better when removing the largest outlier DPA (2,4,6-trinitro-N-(2,4,6-trinitrophenyl)aniline, pred. −3.32, exp. 0.90). In this case, the correlation coefficient of the dataset improves to $r^2 = 0.93$ with a deviation of $rmsd = 0.93$ $\log_{10}$ units. Note that this compound is no outlier at the TZVPD-FINE level, which demonstrates a better handling of anilines by the latter.

In general, both the TZVPD-FINE and the TZVP level could be used for calculating permeability values of anions. By combining the datasets of neutral compounds and anions at the TZVPD-FINE level, slopes close to one (0.99) and intercepts close to zero (−0.06) are obtained, illustrating the predictive character of COSMO*perm* without the requirement for a system-specific calibration, while it must be admitted that the almost exact slope of 0.99 might be a lucky coincidence. The TZVP level turned out to be slightly more predictive, as it did not require the push-pull-effect correction.

A slight non-linearity might occur in the close inspection of **Figure 3** at the low and high permeability ends. This might be related to both the predictions as well as difficulties in the measurements at boundaries of the permeability scale. Because the exact reason is not known, we refrained to apply a polynomial fit; nevertheless, it could be used to improve the statistics as part in further model refinements.

To the best of our knowledge, this is the first mechanistic membrane permeability model, on the one hand operating at the atomistic level of phospholipid membranes, and on the other hand leading to an accuracy of better than one logarithmic unit for both neutral compounds and ions. Even more, the calculation times are in the range of a few minutes, with the potential to apply

COSMO*perm* in both screening applications and detailed investigations of membrane permeabilities.

Permeability of Cations. There is a sparse availability of data for the permeability of cations, because cations traverse the membrane orders of magnitude slower than structurally similar anions due to the positive membrane dipole potential.[35] The few available permeation data differ widely between different assays, as for cations various membrane types are used that often show an increased membrane permeability, to compensate for the decrease in the electrical signal of experimental measurements. Herein, the membrane systems for cations are composed of: (a) asolectin; (b) pure 1,2-di-O-phytanyl-*sn*-glycero-3-phosphocholine (DPhytanylPC), or (c) with additional 5 µM phloretin; and (d) 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine or 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DPhPC/DOPC). The increase of the membrane permeability is probably related to the decrease of the membrane dipole potential. For example, DPhytanylPC contains ether linkages, and the absence of carbonyl groups causes a decrease in the dipole potential of more than 100 mV as compared to membranes formed from lipids containing ester linkages.[51,52] Our analysis of COSMO*perm* performance in predicting cationic permeability data is in consequence mainly based on the systematic measurements of cationic permeability of tetraphenylphosphonium and six of its analogues[53], and of *n*-dodecyltriphenylphosphonium and seven of its analogues[51].

In an initial analysis, all cation-specific membrane types (except DPhPC/DOPC) are used in a common dataset compilation and correlated to COSMO*perm* predictions with the full DMPC membrane (see **Figure 4a**). The slope turns out to be close to one (0.95), but the correlation

coefficient rather moderate ($r^2 = 0.77$), especially related to cations at the high permeability end. Nevertheless, the agreement to the asolectin assay could be interpreted as rather good ($r^2 = 0.91$).

In a second attempt, the membrane layers between the two proposed resistance wells, i.e., two times the innermost 12 layers are used to calculate the membrane permeability ($P = \frac{1}{2\Sigma_{i=1}^{12}R_i}$). The reason is to avoid the influence of headgroups potentially overruling the membrane core (see **Figure S4.2**). Indeed, the qualitative differences in membrane core resistance are captured quite well between compounds in correlation to experimental permeability (see **Figure 4b**). Thus, COSMO*perm* seems to underestimate the cation resistance in the membrane core systematically, and to rather overestimate the resistance in the headgroups. There are several reasons, which might explain this effect:

(1) The membrane dipole potential has been fitted to reproduce membrane/water partition coefficients (log $K_{m:w}$) The shape of this potential might be less sensitive to both the prediction of log $K_{m:w}$ values and the prediction of anion permeabilities than to the prediction of cation permeabilities. Note that the dipole potential is used in an entirely predictive manner for COSMO*perm* and has not been refitted herein. See section S3 in the supporting information for further details.

(2) The calculated conformer set contains mainly stretched conformers for these large dodecylphosphonium derivatives. This is not relevant in classic COSMO-RS bulk phase calculations; therefore a large portion of tilted conformers is typically dropped by the COSMO*conf* conformer generation algorithm applied, unless they had an effect on the chemical potential. However, tilted conformers might contribute significantly to the properties of inhomogenous systems.[21]

(3) The dodecylphosphonium derivatives have a similar extension as the membrane itself, potentially hitting the limitation of the infinite-dilution limit of the classic COSMO*mic* approach. Also, their sizes might be limiting the calculation, because they could potentially disturb the membrane structure. This could be overcome by a combined application of COSMO*plex* and COSMO*perm* to reach finite concentration effects, which is beyond the scope of this manuscript.

(4) The free energy profiles are probably influenced by salt or buffer concentrations in the aqueous phase, including potential protonation or deprotonation reactions close to the zwitterionic headgroups, which are not considered at the current stage.

While Rokitskaya *et al.* did not observe a correlation between hydrophobicity and membrane permeability, lower COSMO*perm* resistances in the membrane core are directly related to higher experimental membrane permeabilities, and thus suggest a correlation between membrane permeability and compound hydrophobicity. The only exception to this rule are the three most hydrophobic dodecyltriphenylphosphonium derivatives (tri(3,5-dimethylphenyl), tri(2,4,6-trimethylphenyl) , trinaphthyl). The measured permeabilities are underestimated, possibly because membrane permeability may already have reached a decreasing range at the measured concentration of 0.1−1.8 μM; and saturation effects seem to set in at lower compound concentration the higher the membrane permeability of a compound.[31] The three values were thus not considered in the following fit.

To address the systematic overestimation of membrane permeability, we fitted a linear correlation to each group of data points from literature measured in the different membrane types mentioned above. We assumed a systematic shift between different lipid groups, most likely due to a change in membrane dipole potential, and thus applied a membrane specific shift along with a global fit of the slope that is in best agreement with data points for all four assays (a-d). For the

DPhPC/DOPC membranes, only permeability data for two different compounds were available, therefore the correlation for this membrane system, which should resemble that of our DMPC predictions most, is fit by using the fixed slope extracted from the other three curves. These correlations, stated in **Figure 4b**, may be used to predict cationic membrane permeability for a respective membrane type.

pH Dependence of Permeability. The pH dependence of the predicted salicylic acid permeability is compared to BLM values in the literature. [27] The pH dependence of individual protonation states, predicted by COSMO*perm*, is then calculated by using the predicted BLM values in **equation 9**, and eventually the total pH dependent BLM permeability of all protonation states by **equation 8**. The good agreement of the COSMO*perm* model with the experiment is shown in **Figure 5**. Slope and intercept are almost one and zero, respectively, and the *rmsd* value as low as $0.08 \log_{10}$ units.

Note that the experimental values refer to the intrinsic membrane permeability; the unstirred water layer has been deducted from these values. An accurate description of the unstirred water layer is potentially adjuvant for many permeability assays (e.g., BLM, Caco-2, PAMPA and several more);[3] however, this is beyond the scope of this manuscript focusing on the intrinsic membrane permeability alone. This topic will be further investigated in upcoming publications.

Permeability in Different Membrane Systems. All results presented up to now are validated against classic phospholipid membranes, mostly stabilized by viscous, non-polar alkane solvents in the experiments. One strength of the COSMO*perm* method is its applicability to membrane systems in general, as long as two conditions are met: First, an atomistic membrane

structure can be generated, and second, the membrane system is at the liquid-crystalline state (for the validity of the fluid phase COSMO-RS equations).[12]

To illustrate this general applicability, a DMPC and chlorodecane containing membrane at the interface to water has been generated using COSMO*plex*. The experimental validation dataset is extracted from the supporting information of Ebert *et al.*[31] and shown in **Table 3**. The predicted COSMO*perm* values of the chlorodecane containing system are compared to the predicted values of the pure DMPC system (additional experimental and predicted values for the chlorodecane/decane/phospholipid system are listed in **Table S5.1** of the supporting information). On average, the permeability values are two to three orders of magnitude higher in the chlorodecane containing system (mean: −3.33; median: −3.04), a consequence of the increase of the dielectric constant inside the membrane core due to the chlorodecane.

A similar decrease of the membrane barrier for chlorodecane containing BLM by about three orders of magnitude is reported several times in the literature.[31,54] Chlorodecane containing membranes are used as a reference system for mitochondrial membranes, as their anionic permeability was shown to be very similar.[54,55] The reason for this similarity is believed to lie in the high amount of proteins present in the mitochondrial membrane, which supposedly causes a similar change in dielectric constant as chlorodecane.[54]

Further investigations will include more complex cell plasma membrane structures, which are formed by a complex mixture of phosphatidylcholines, -serines, and -ethanolamines, sphingomyelin, glycolipids and especially a varying amount of cholesterol.[56] These membrane structures could be generated using the COSMO*plex* method[57] and the corresponding solute permeabilities predicted by COSMO*perm*.

CONCLUSIONS

Because of its generality, good predictivity and sound mechanistic treatment, the COSMO*perm* approach is a valuable tool for predicting the membrane permeability for a wide variety of neutral and ionic organic compounds. In addition, the $\Delta G(z)$ and $D(z)$ profiles are obtained quickly as a matter of a few minutes, as compared to time-consuming molecular dynamics simulations. This model allows for the examination of pH dependent effects on the passive permeation through phospholipid membranes, where these effects are captured on an atomic scale in a fully mechanistic way. The COSMO*perm* approach can be generally applied to any kind of membranes without the need for a re-calibration, as demonstrated by the artificial chlorodecane containing phospholipid system (generated by the COSMO*plex* method), or further possibilities, including cholesterol containing systems or skin lipid systems (e.g., in the *stratum corneum*).[57] Additional investigations include the integration of the COSMO*perm* approach into more complex systems involving aqueous boundary layers or living cell systems (e.g., Caco-2). The currently present infinite dilution approximation of COSMO*perm* can be overcome by a combination of the COSMO*perm* and COSMO*plex* approaches. The latter has extended the original COSMO*mic* applicability domain to finite concentration ranges. COSMO*perm* is designed and works well for membranes at the liquid-crystalline state. Further investigations are required, and additional terms need to be implemented for a proper description at the gel-phase state below the phase transition temperature.

ASSOCIATED CONTENT

Supporting Information. The supporting information contains details of the diffusion coefficient model, calculation of fractions of individual protonation states, details on the membrane models, and additional cation and chlorodecane containing membrane data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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Schwöbel and Uwe Huniar are employees of Dassault Systèmes. Dassault Systèmes commercially

distributes the BIOVIA COSMO*therm*, BIOVIA COSMO*conf* and TURBOMOLE software

packages used in this paper, including the COSMO*perm* and COSMO*plex* extensions.

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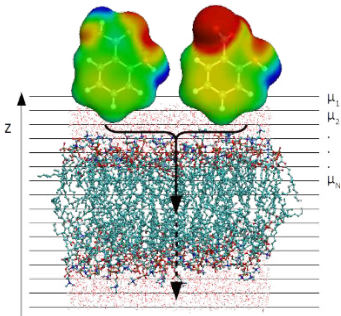
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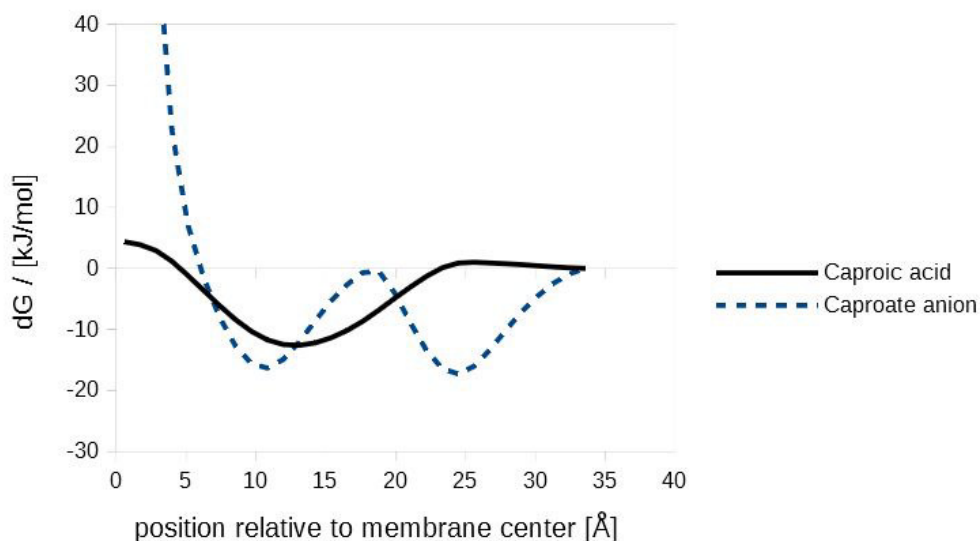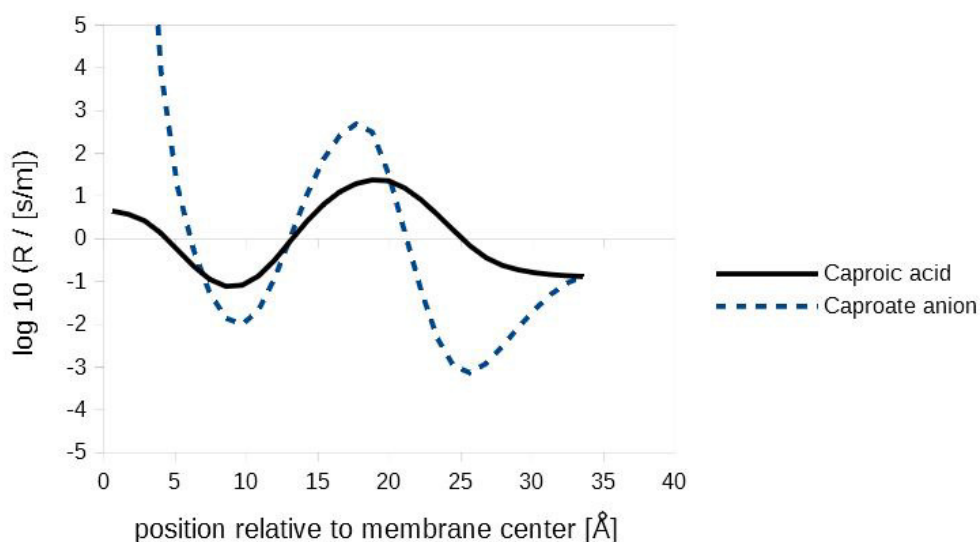
TOC Graphics

Figure 1. COSMO*perm* free energy profiles of caproic acid (black line) and the corresponding caproate anion (blue line): a) free energy profiles in [kJ/mol] units, top; b) resistance profiles in [s/m] units, bottom. On the left hand side is the central alkyl part of the membrane, on the right hand side the water phase.
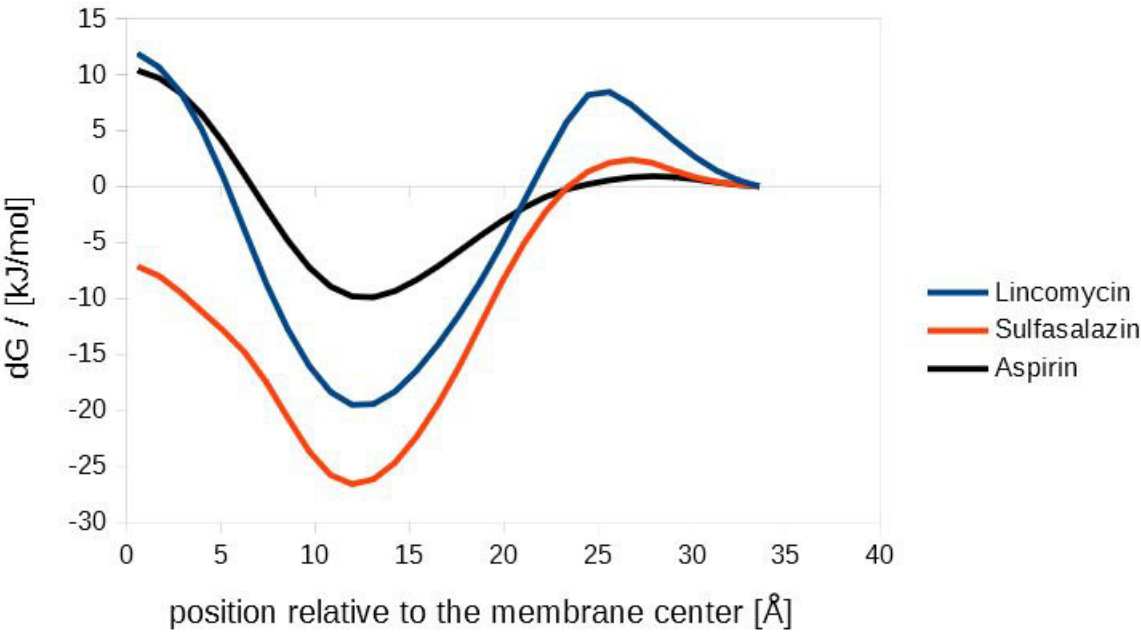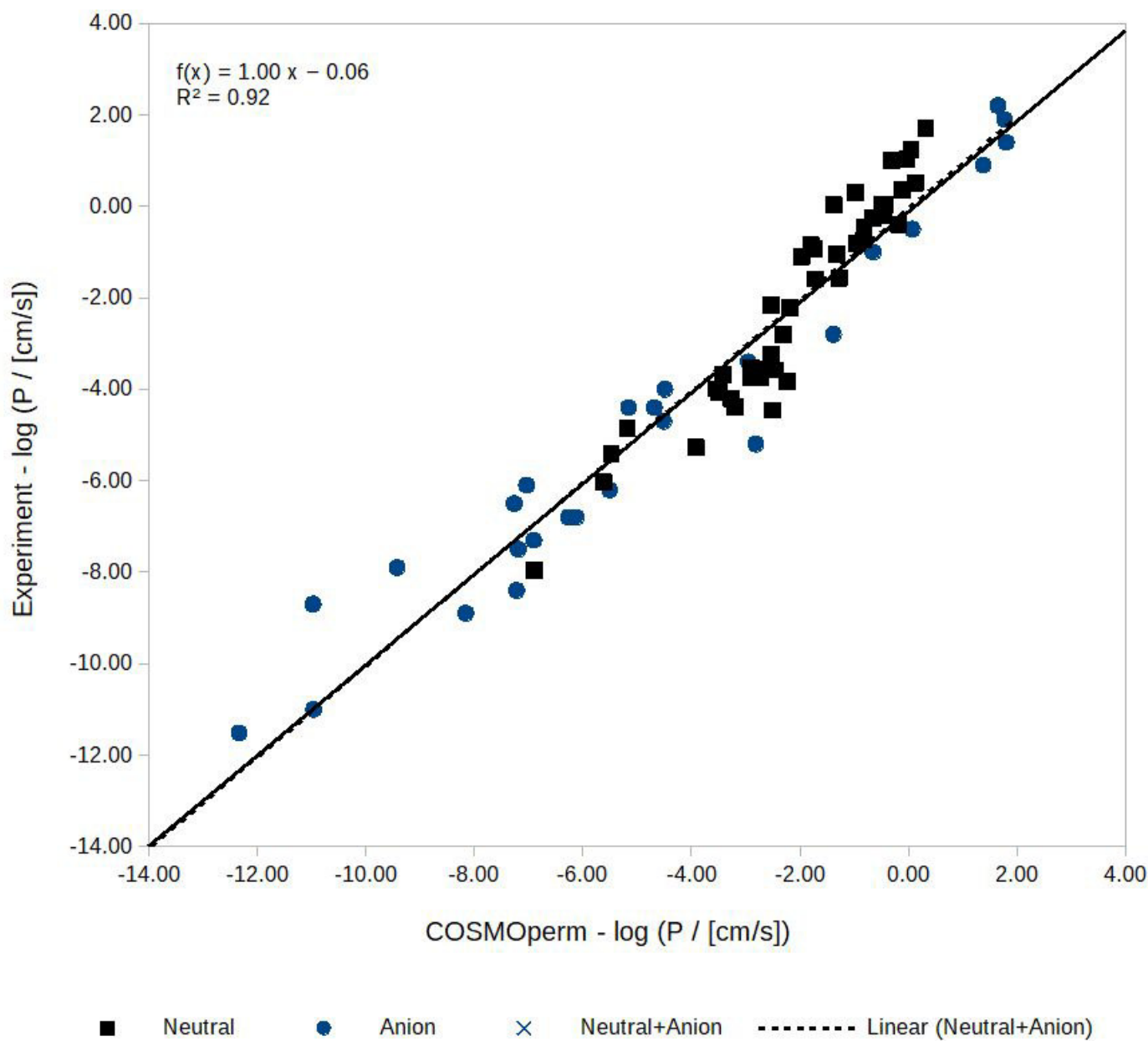
Figure 1a.

Figure 1b.

Figure 2. COSMO*perm* free energy profiles in [kJ/mol] profiles of aspirin, sulfasalazine and lincomycin (black line, red line and blue line, respectively) with a significant second energy barrier in the polar head group region, in addition to the membrane interior.
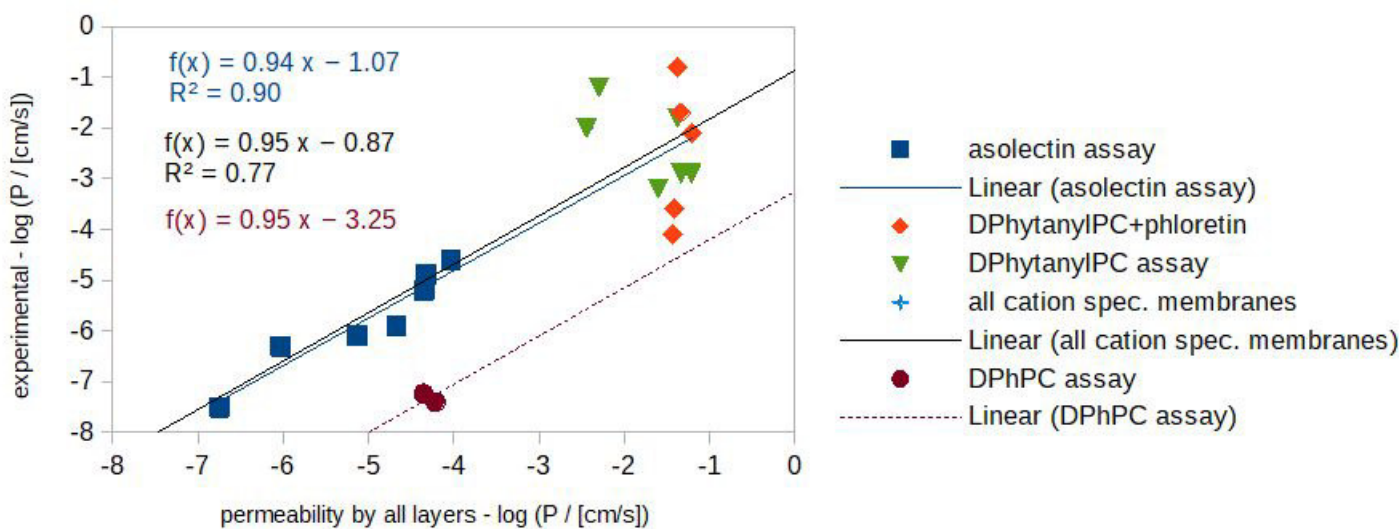
Figure 3. Experimental permeabilities and permeabilities predicted by COSMO*perm*; neutral compounds in black (square), anions in blue (circles).
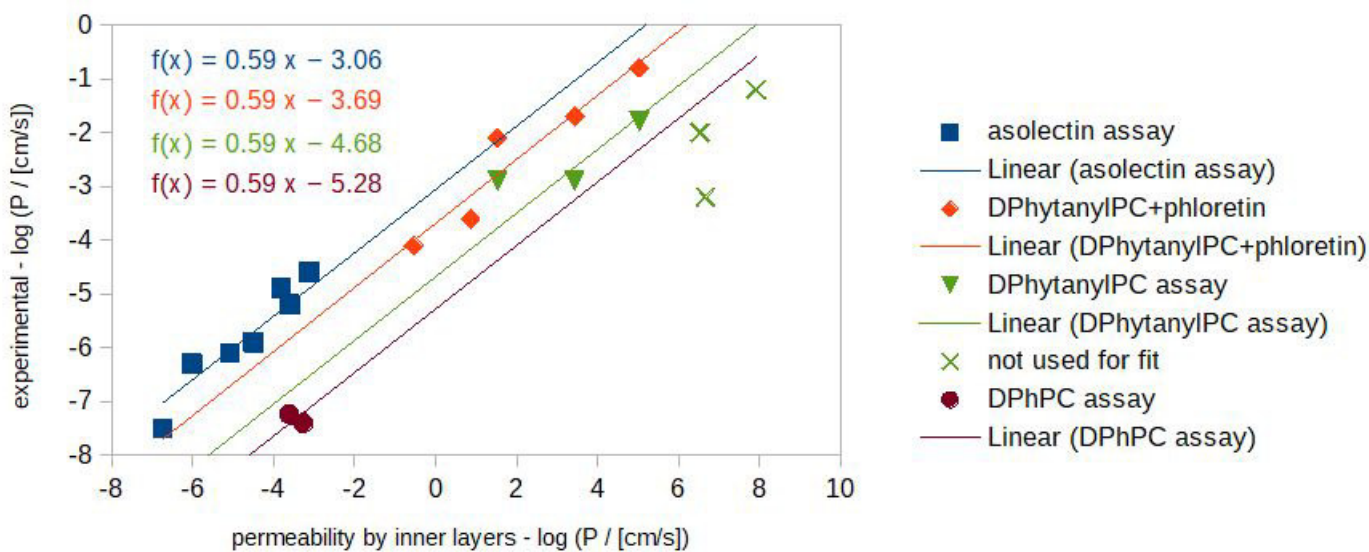
Figure 4. Experimental and predicted cation permeabilities for a) all layers (top); or b) for the well-to-well, innermost 24 membrane layers (bottom). The slope of 0.59 was extracted by a global linear fit, and the individual offsets of the depicted correlations correct for the overestimation of membrane permeability depending on the membrane type. Crossed symbols were not considered in the fit (see text).
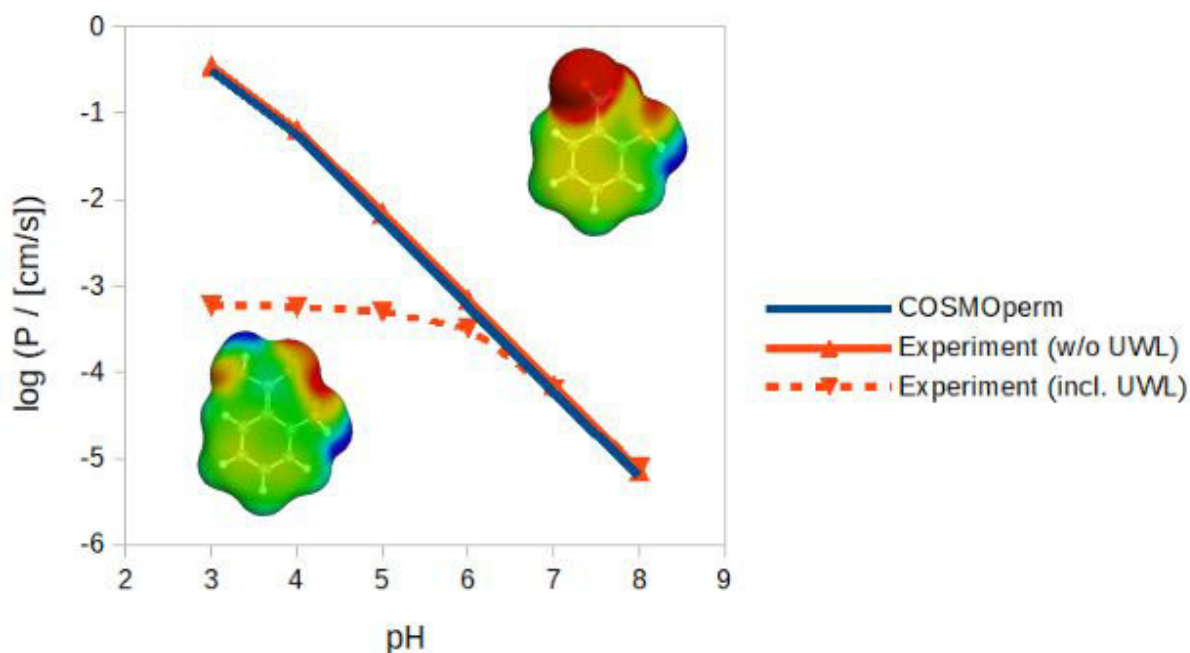
Figure 4a.

Figure 4b.

Figure 5. pH dependence of experimental effective permeabilities for salicylic acid,[27] and corresponding permeabilities predicted by COSMO*perm*. The dotted line shows the experimental pH dependence derived by the ion flux, which is not corrected for effects introduced by the unstirred water layer (UWL). The COSMO surfaces are shown for the neutral (left) and deprotonated state (right), keeping in mind that the permeability value is dominated by the neutral state at all pH values.

Table 1. Set of Experimental ("Exp.") and Predicted ("Pred.") Phospholipid Bilayer Membrane Permeabilities for Neutral Compounds and Ions in $\log_{10}([cm/s])$ units.

Name	Neutral log (P / [cm/s])			Ion log (P / [cm/s])					
	Exp.[a]	TZVPD-FINE	TZVP	Exp. Anion[b]	Exp. Cation[c]	Pred.[d]	TZVPD-FINE	TZVP	Push-Pull
1,2-ethanediol	−4.1	−3.5	−3.2						
1,2-propanediol	−3.6	−2.7	−2.3						
1,4-butanediol	−3.6	−2.4	−2.4						
2-(2-methyl-2-propanyl)-4,6-dinitrophenol (*dino2terb*)		−0.3	0.4	−4.0		−4.5	−2.0	−4.0	yes
2-butan-2-yl-4,6-dinitrophenol (*dinoseb*)		−0.3	0.5	−4.4		−5.2	−2.7	−4.6	yes
2,4-dinitrophenol		−0.6	−0.1	−6.5		−7.3	−4.8	−6.8	yes
2,4,6-tribromophenol		−0.1	0.4	−7.3		−6.9	−6.9	−6.4	no
2,4,6-trinitrophenol	−0.4[q]	−0.2	0.4	−4.1		−6.1	−3.6	−3.6	yes
2,4,6-trinitro-N-(2,4,6-trinitrophenyl)aniline (*DPA*)		0.1[r]	1.6	0.9		1.4	1.4	−3.3	no
2'-deoxyadenosine	−6.0	−5.6	−4.8				−17.8	−12.6	no
2',3'-dideoxyadenosine	−4.2	−3.3	−3.0				−11.8	−9.9	no
3,4-dinitrophenol		−1.4	−0.2	−7.5		−7.2	−4.7	−6.3	yes
3,5-dibromo-2-(2,4-dibromophenoxy)phenol		0.6	1.5	−5.2		−2.8	−2.8	−3.5	no
3,5-dichlorophenol		0.1	0.4	−7.9		−9.4	−9.4	−9.0	no
4-nitro-2-(trifluoromethyl)-benzimidazol		−1.3	−0.8	−6.1		−7.0	−4.6	−5.9	yes
4-nitrophenol		−1.2	−0.6	−8.9		−8.2	−5.7	−9.5	yes
5,6-dichloro-2-(trifluoromethyl)-1H-benzimidazole (*DTFB*)	0.3[m]	−1.0	−0.4				−8.1	−9.1	no
9-anthracenecarboxylic acid	0.5	0.1	0.4				−12.8	−15.0	no
acetamide	−3.7	−3.4	−4.0						
acetic acid	−2.2	−2.2	−2.0				−19.8	−21.8	no
adenine	−4.9	−5.2	−4.7				−14.2	−12.7	no
alpha-carbamoyl-*p*-toluic acid	−4.4	−3.2	−3.7				−9.7	−17.0	no
alpha-carboxy-*p*-toluic acid	−3.7	−2.9	−2.2				−11.1	−16.1	no
alpha-chloro-*p*-toluic acid	−0.2	−0.5	−0.2				−14.1	−15.5	no
alpha-cyano-*p*-toluic acid	−1.6	−1.3	−1.5				−12.1	−16.2	no
alpha-hydroxy-*p*-toluic acid	−2.8	−2.3	−1.9				−14.9	−17.4	no
alpha-methoxy-*p*-toluic acid	−0.5	−0.8	−0.5				−14.8	−16.3	no
alpha-naphthoic acid	0.4	−0.1	0.0				−14.4	−16.4	no
ascorbic acid	−8.0[e]	−6.9	−5.2	≤−11.5[e]		−12.3	−12.3	−14.7	no
aspirin	−0.8[f]	−1.0	−0.6				−15.0	−16.5	no
benzoic acid	−0.3	−0.7	−0.5				−16.7	−17.8	no

beta-naphthoic acid	1.2	0.0	0.1				−14.7	−16.1	no
bis(fluorosulfonyl)amide		0.0	0.5	−4.7		−4.5	−4.5	−2.4	no
bromoxynil		−0.6	−0.3	−6.2		−5.5	−3.0	−6.1	yes
butyltriphenylphosphonium					−5.9	−5.8	−4.7		no
butyric acid	−1.1	−1.3	−1.0				−18.5	−20.2	no
carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (*FCCP*)	1.7[j]	0.3	0.0				−1.8	−2.7	no
carbonylcyanide *m*-chlorophenylhydrazone (*CCCP*)	1.0[l]	0.0	−0.3	−3.4		−3.0	−3.0	−3.4	no
codeine	−0.9	−1.8	−1.2				−14.8	−7.9	no
coumachlor		−1.1	−0.2	−6.8		−6.1	−3.6	−7.1	yes
cyano(triphenyl)boranuide			−2.2	−2.8		−1.4	−1.4	−3.2	no
ethylamine	−0.9[g]	−1.7	−2.1				−16.7	−12.9	no
ethyltriphenylphosphonium					−6.3	−6.7	−6.0		no
flufenamic acid		0.4	0.9	−6.8		−6.3	−6.3	−9.4	no
formamide	−4.0[h]	−3.5	−4.4						
formic acid	−2.2[i]	−2.5	−2.7				−18.5	−20.9	no
glycerol	−5.3	−3.9	−3.3						
hexanoic acid	0.0	−0.5	−0.3				−17.1	−19.0	no
hexyl(triphenyl)phosphonium					−4.6	−4.9	−4.0		no
histamine	−4.5	−2.5	−2.9				−13.8	−6.7	no
hydrocortisone	−3.3	−2.5	−1.5						
hydrocortisone-21-pimelamide	−3.7	−2.7	−2.9					−10.9	no
methylamine	−1.1[g]	−2.0	−2.4				−17.3	−14.4	no
methyltriphenylphosphonium					−7.5	−7.1	−6.7		no
n-dodecyl trinaphthyl phosphonium					−3.2[p]	−0.7	−1.6		no
n-dodecyl tri(3,5-dimethylphenyl) phosphonium					−1.2[p]	0.1	−2.3		no
n-dodecyl tri(*p*-chloro-phenyl) phosphonium					[−3.6][p]	[−3.2]	−1.4		no
n-dodecyl tri(*p*-fluorophenyl) phosphonium					[−4.1][p]	[−4.0]	−1.4		no
n-dodecyl tri(*p*-methoxyphenyl) phosphonium					−2.9[p] [−1.7]	−2.6 [−1.6]	−1.3		no
n-dodecyl tri(*p*-tolyl) phosphonium					−1.8[p] [−0.8]	−1.7 [−0.7]	−1.4		no
n-dodecyl triphenyl phosphonium					−2.9[p] [−2.1]	−3.8 [−2.8]	−1.2		no
n-dodecyl tri(2,4,6-trimethylphenyl) phosphonium					−2.0[p]	−0.8	−2.4		no
p-toluic acid	0.0	−0.4	−0.2				−16.8	−17.6	no
pentachlorophenol		0.0	0.5	−4.4		−4.7	−4.7	−4.6	no
perchloric acid		−1.0	−0.5	−8.7		−11.0	−11.0	−8.1	no
phloretin	−3.6	−2.8	−1.4				−8.2	−9.7	no
prednisolone	−3.8	−2.2	−1.8						

propionic acid	−1.6	−1.7	−1.4				−19.0	−20.8	no
salicylic acid	−0.1	−0.5	0.0	−11.0		−11.0	−11.0	−12.5	no
tetrachlorotrifluoromethylbenzimidazole (*TTFB*)	1.0[k]	−0.3	0.2						
tetraethylboranuide				−1.0		−0.7	−0.7	−0.3	no
tetrakis(3-trifluoromethylphenyl)boranuide				2.2		1.6	1.6	1.8	no
tetrakis(4-chlorophenyl)boranuide				1.9		1.8	1.8	1.8	no
tetrakis(4-fluorophenyl)boranuide				1.4		1.8	1.8	1.8	no
tetraphenylarsonium					−7.2[n]	−7.2	−4.2		no
tetraphenylborate				−0.5		0.1	0.1	0.8	no
tetraphenylphosphonium					−5.2[o]	−5.2	−4.0	−2.6	no
theophylline	−3.5	−2.9	−3.3				−8.8	−13.5	no
triethylamine	0.0	−1.4	−0.8				−14.5	−4.8	no
triphenylamylphosphonium					−4.9	−5.4	−4.3		no
triphenylpropylphosphonium					−6.1	−6.1	−5.1		no
tryptamine	−0.7	−0.8	−1.1				−14.4	−11.0	no
Urea	−5.4	−5.5	−6.4						
warfarin		−1.1	−0.4	−8.4		−7.2	−4.7	−8.3	yes

Table 2. COSMO*perm* Statistics for Different Datasets.

Dataset	Model Membrane	TZVPD-FINE						TZVP				
		N	r^2	*rmsd*	*slope*	*intercept*	*rmsd^a*	r^2	*slope*	*intercept*	*rmsd*	*rmsd^a*
Neutral	DMPC	45	0.91	0.84	1.29	+0.56	0.69	0.81	1.21	+0.17	1.06	0.98
Anions[b]	DMPC	27	0.94	1.00	0.90	−0.47	0.93	0.89 [0.93]	0.83	−0.81	1.40 [1.17]	1.26 [0.93]
Neutral + Anions[b]	DMPC	72	0.92	0.90	0.99	−0.06	0.90	0.87 [0.90]	0.92	−0.39	1.20 [1.10]	1.17 [1.06]
Cations[c]	DMPC	16	0.81	NA	0.95	−0.87	0.93					
Cations[d]	DMPC (inner layers)	16	0.90	NA	0.59	assay dependent	0.82					
Selected anions	DMPC+ chlorodecane	6	0.96	1.57	0.81	+0.69	0.52	0.92	1.07	+0.09	0.88	0.88

ᵃ Corrected for slope and intercept; [b] Values in brackets without DPA, the largest outlier at TZVP level; [d] Cation data measured by a set of assays. The r^2 and *rmsd* values shown use a slope of 0.95 and an intercept of −0.87 for all membranes adapted to cations and −3.25 for DPhPC/DOPC membranes (see **Figure 4a**) [d] Cation data measured by a set of assays. The r^2 and *rmsd* values shown use a slope of 0.59 and assay dependent intercepts (see **Figure 4b**).

Table 3. Set of Experimental Ions Permeabilities in $\log_{10}$(cm/s) units in a Chlorodecane/DMPC System, Predicted Permeabilities in a Chlorodecane/DMPC Model System (TZVP) and Differences to the DMPC Model System (TZVP).

Compound[a]	Permeability in $\log_{10}$(cm/s)			
	Experiment[b]	DMPC+ Chlorodecane	DMPC (pure)	Difference
CCCP anion	-0.50	-1.05	-3.39	-2.34
DTFB anion	-1.50	-1.16	-3.89	-2.73
FCCP anion	0.30	-0.42	-2.70	-2.28
perchlorate anion	-5.80	-3.95	-8.12	-4.17
S-13 anion	0.80	0.79	-2.56	-3.35
thiocyanate anion	-5.80	-6.36	-11.49	-5.13

[a] Abbreviations are listed in **Table 1**; S-13: 3-tert-butyl-5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide. [b] Ref. ([31]), table S7, additional data listed in the supporting information.